## STATE OF MISSOURI

## DEPARTMENT OF NATURAL RESOURCES

## MISSOURI CLEAN WATER COMMISSION



# MISSOURI STATE OPERATING PERMIT

In compliance with the Missouri Clean Water Law, (Chapter 644 R.S. Mo. as amended, hereinafter, the Law), and the Federal Water Pollution Control Act (Public Law 92-500, 92<sup>nd</sup> Congress) as amended,

Permit No.:	MO-0000353
Owner: Address:	Union Electric Company dba AmerenUE One Ameren Plaza, PO Box 66149 (MC 602), St. Louis, MO 63166
Continuing Authority: Address:	Same as above Same as above
Facility Name: Address:	AmerenUE, Sioux Power Plant 8501 North State Route 94, West Alton, MO 63386
Legal Description:	USG Survey 1838, T48N, R6E, St. Charles County
Receiving Stream: First Classified Stream and ID: USGS Basin & Sub-watershed No.:	Mississippi River (P) Mississippi River (P)(00001) (07110009-030004)
is authorized to discharge from the fac as set forth herein:	ility described herein, in accordance with the effluent limitations and monitoring requirements
FACILITY DESCRIPTION	
See page 2	
	er discharges under the Missouri Clean Water Law and the National Pollutant Discharge to other regulated areas. This permit may be appealed in accordance with Section 644.051.6 of
April 16, 2004 Effective Date	Stephen M. Mahfooli, Director Department of Natural Resources Executive Secretary, Clean Water Commission
April 15, 2009	

Jim Hull, Director of Staff, Clean Water Commission

Expiration Date

MO 780-0041 (10-93)

## FACILITY DESCRIPTION (continued)

Outfall #001 - Power Plant - SIC #4911

Non-contact cooling water discharge.

Design flow is 1,344 MGD.

Actual flow is 863 MGD.

Outfall #002 - Power Plant - SIC #4911

Ash pond/pH neutralization.

Design flow is 16.662 MGD.

Actual flow is 4.855 MGD.

## Outfall #002A - Power Plant - SIC #4911

Flow equalization/extended aeration/sludge holding tank/sludge disposal is by contract hauler.

Design population equivalent is 170.

Design flow is 0.039 MGD.

Actual flow is 0.005 MGD.

Design sludge production is 3.06 dry tons/year.

#### Outfall #003 - Power Plant - SIC #4911

Emergency overflow from the combined drain sump.

Design flow is 3.48 MGD.

#### Outfalls #004 & #005 - Power Plant - SIC #4911

These outfalls still exist but are not currently being monitored due to Implementation of Best Management Practices & minimal risk to waters of the state.

Outfall #006 - Power Plant - SIC #4911

Ash pond #2/pH neutralization.

Design flow is 13.656 MGD.

Actual flow is 5.64 MGD.

This outfall will receive storm water from chipped tire pile.

## PAGE NUMBER 3 of 11

## A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

PERMIT NUMBER MO-0000353

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

OUTEAU NUMBER AND EEELUENT		FINAL EFFLUENT LIM			MONITORING REQUIREMENTS	
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #001 - Non-Contact	Cooling W	ater				
Flow	MGD	*		*	once/weekday**	24 hr. estimate
Intake Water Temperature	٥F	*		*	once/weekday**	grab
Effluent Temperature	٥F	*		*	once/weekday**	grab
Thermal Discharge (Internal Energy Increase)	btu/hr	5.50 x 10 <sup>9</sup>			once/weekday**	grab
MONITORING REPORTS SHALL BE SU	JBMITTED MC	NTHLY; THE FIR	ST REPORT	IS DUE Jur	ne 28, 2004.	
Whole Effluent Toxicity % (WET) Test	Survival	ival See Special Condition #1			once/year	grab
MONITORING REPORTS SHALL BE SU	JBMITTED AL	NUALLY; THE FI	RST REPOR	T IS DUE O	ctober 28, 2004.	
Outfall #002 - Ash Pond						
Flow	MGD	*		*	once/week	24 hr. estimate
<pre>Intake Total Suspended Solids***</pre>	mg/L	*		*	once/week	grab
Effluent Total Suspended Solids***	mg/L	*		*	once/week	grab
Net Total Suspended Solids	mg/L	100		30	once/week	grab
Oil and Grease	mg/L	20		15	once/month	grab
pH - Units	SU	***		****	once/week	grab
MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE $\underline{\text{June 28, 2004}}$ .						
Sulfate (as $SO_4^{-2}$ )	mg/L	*		*	once/quarter***	*** grab
MONITORING REPORTS SHALL BE SU	JBMITTED QT	JARTERLY; THE I	FIRST REPO	RT IS DUE	July 28, 2004.	
Whole Effluent Toxicity % (WET) Test	Survival	See Special Condition #1			once/year in January	grab

MONITORING REPORTS SHALL BE SUBMITTED ANNUALLY; THE FIRST REPORT IS DUE  $\underline{\text{October}} 28$ ,  $\underline{2004}$ . THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

## **B. STANDARD CONDITIONS**

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED  $\underline{\texttt{Parts}}$  I & III STANDARD CONDITIONS DATED  $\underline{\texttt{October}}$  1, 1980 and  $\underline{\texttt{August}}$  15, 1994, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

## A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

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PERMIT NUMBER MO-0000353

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

		FINAL EF	FLUENT LIMI	TATIONS	MONITORING REQUIREMENTS			
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT S FREQUENCY	SAMPLE TYPE		
Outfall #002A - Sewage Treatment Plant								
Flow	MGD	*		*	once/month	24 hr. estimate		
Biochemical Oxygen Demands	mg/L		45	30	once/quarter****	****		
Total Suspended Solids	mg/L		45	30	once/quarter****	****		
pH - Units	SU	***		****	once/quarter****	grab		

MONITORING REPORTS SHALL BE SUBMITTED QUARTERLY; THE FIRST REPORT IS DUE July 28, 2004.

Outfall #003 - Emergency Overflow From Sump

Flow	MGD	*	*	once/day when 24 hr. discharge occurs estimate
Total Suspended Solids	mg/L	100	30	once/day when grab discharge occurs
Oil and Grease	mg/L	20	15	once/day when grab discharge occurs
pH - Units	SU	***	****	once/day when grab discharge occurs

MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE  $\underline{\text{June}}$  28, 2004. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

## **B. STANDARD CONDITIONS**

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED  $\underline{\texttt{Parts}}$  I & III STANDARD CONDITIONS DATED  $\underline{\texttt{October}}$  1, 1980 and  $\underline{\texttt{August}}$  15, 1994, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

MO 780-0010 (8/91)

## A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

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PERMIT NUMBER MO-0000353

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

		FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #006 - New Ash Pon	d					
Flow	MGD	*		*	once/week	24 hr. total
Intake Total Suspended Solids***	mg/L	*		*	once/week	grab
Effluent Total Suspended Solids***	mg/L	*		*	once/week	grab
Net Total Suspended Solids***	mg/L	100		30	once/week	grab
Oil and Grease	mg/L	20		15	once/month	grab
pH - Units	SU	***		***	once/week	grab
MONITORING REPORTS SHALL BE S	SUBMITTED MONT	THLY; THE FIR	ST REPORT	IS DUE Jun	e 28, 200 <u>4</u> .	
Sulfate (as ${\rm SO_4}^{-2}$ ) mg/L		*		*	once/quarter*	**** grab
MONITORING REPORTS SHALL BE S	SUBMITTED QUAF	TERLY; THE	FIRST REPO	RT IS DUE J	uly 28, 2004.	
Whole Effluent Toxicity (WET) Test	% Survival	See Special Condition #1			once/year in January	grab
MONITORING REPORTS SHALL BE S	SUBMITTED ANNU	JALLY; THE F	IRST REPOR	T IS DUE Oc	tober 28, 2004.	THERE

MONITORING REPORTS SHALL BE SUBMITTED ANNUALLY; THE FIRST REPORT IS DUE October 28, 2004. THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

## **B. STANDARD CONDITIONS**

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED  $\underline{\texttt{Parts}}$  I & III STANDARD CONDITIONS DATED  $\underline{\texttt{October}}$  1, 1980 and  $\underline{\texttt{August}}$  15, 1994, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

MO 780-0010 (8/91)

#### A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS (continued)

- \* Monitoring requirement only.
- \*\* Sample once a weekday means: Monday, Tuesday, Wednesday, Thursday, and Friday.
- \*\*\* Intake Non-Filterable Residue (Total Suspended Solids) values may be used to calculate "net" limitations. However, the permittee must continue to maintain the ash pond system for adequate retention time for settling. River solids present in intake water are "treated" in the ash pond system but treatment levels are dependent on concentration and types of river solids present in intake water.
- \*\*\*\* pH is measured in pH units and is not to be averaged. The pH is limited to the range of 6.0-9.0 pH units.
- \*\*\*\* Sample once per quarter in the months of February, May, August, and November.
- \*\*\*\*\* A composite sample made up from a minimum of four grab samples collected within a 24-hour period with a minimum of two hours between each grab sample.

#### C. SPECIAL CONDITIONS

- 1. This permit may be reopened and modified, or alternatively revoked and reissued, to:
  - (a) Comply with any applicable effluent standard or limitation issued or approved under Sections 301(b)(2)(C) and (D), 304(b)(2), and 307(a) (2) of the Clean Water Act, if the effluent standard or limitation so issued or approved:
    - (1) contains different conditions or is otherwise more stringent than any effluent limitation in the permit; or
    - (2) controls any pollutant not limited in the permit.
  - (b) Incorporate new or modified effluent limitations or other conditions, if the result of a waste load allocation study, toxicity test or other information indicates changes are necessary to assure compliance with Missouri's Water Quality Standards.
  - (c) Incorporate new or modified effluent limitations or other conditions if, as the result of a watershed analysis, a Total Maximum Daily Load (TMDL) limitation is developed for the receiving waters which are currently included in Missouri's list of waters of the state not fully achieving the state's water quality standards, also called the 303(d) list.

The permit as modified or reissued under this paragraph shall also contain any other requirements of the Clean Water Act then applicable.

- 2. Report as no-discharge when a discharge does not occur during the report period.
- 3. There shall be no discharge of polychlorinated biphenyl compounds.
- 4. Discharge of wastewater from this facility must not alone or in combination with other sources cause the receiving stream to violate the following:
  - (a) Water temperatures and temperature differentials specified in Missouri Water Quality Standards shall be met.
- 5. Any pesticide discharge from any point source shall comply with the requirements of Federal Insecticide, Fungicide and Rodenticide Act, as amended (7 U.S.C. 136 et. seq.) and the use of such pesticides shall be in a manner consistent with its label.
- 6. Neither free available chlorine nor total residual chlorine may be discharged from any unit for more than two hours in any one day.
- 7. An upset provision, identical to the upset provision set forth at 40 CSR 122.41(n), is hereby incorporated in this permit.
- 8. Changes in Discharges of Toxic Substances

The permittee shall notify the Director as soon as it knows or has reason to believe:

- (a) That any activity has occurred or will occur which would result in the discharge of any toxic pollutant which is not limited in the permit, if that discharge will exceed the highest of the following "notification levels:"
  - (1) One hundred micrograms per liter (100  $\mu$ g/L);
  - (2) Two hundred micrograms per liter (200  $\mu g/L$ ) for acrolein and acrylonitrile; five hundred micrograms per liter (500  $\mu g/L$ ) for 2,5 dinitrophenol and for 2-methyl-4, 6-dinitrophenol; and one milligram per liter (1 mg/L) for antimony;
  - (3) Five (5) times the maximum concentration value reported for the pollutant in the permit application;
  - (4) The level established in Part A of the permit by the Director.
- (b) That they have begun or expect to begin to use or manufacture as an intermediate or final product or byproduct any toxic pollutant, which was not reported in the permit application.
- 9. Permittee will cease discharge by connection to areawide wastewater treatment system within 90 days of notice of its availability.

- 10. General Criteria. The following water quality criteria shall be applicable to all waters of the state at all times including mixing zones. No water contaminant, by itself or in combination with other substances, shall prevent the waters of the state from meeting the following conditions:
  - (a) Waters shall be free from substances in sufficient amounts to cause the formation of putrescent, unsightly or harmful bottom deposits or prevent full maintenance of beneficial uses;
  - (b) Waters shall be free from oil, scum and floating debris in sufficient amounts to be unsightly or prevent full maintenance of beneficial uses;
  - (c) Waters shall be free from substances in sufficient amounts to cause unsightly color or turbidity, offensive odor or prevent full maintenance of beneficial uses;
  - (d) Waters shall be free from substances or conditions in sufficient amounts to result in toxicity to human, animal or aquatic life;
  - (e) There shall be no significant human health hazard from incidental contact with the water;
  - (f) There shall be no acute toxicity to livestock or wildlife watering;
  - (g) Waters shall be free from physical, chemical or hydrologic changes that would impair the natural biological community;
  - (h) Waters shall be free from used tires, car bodies, appliances, demolition debris, used vehicles or equipment and solid waste as defined in Missouri's Solid Waste Law, section 260.200, RSMo, except as the use of such materials is specifically permitted pursuant to section 260.200-260.247.
- 11. Sludge and Biosolids Use For Domestic Wastewater Treatment Facilities
  - (a) Permittee shall comply with the pollutant limitations, monitoring, reporting, and other requirements in accordance with the attached permit Standard Conditions.
  - (b) If sludge is not removed by a contract hauler, permittee is authorized to land apply biosolids. Permit Standard Conditions, Part III shall apply to the land application of biosolids. Permittee shall notify the department at least 180 days prior to the planned removal of biosolids. The department may require submittal of a biosolids management plan for department review and approval as determined appropriate on a case-by-case basis.
- 12. Use or Disposal of Ash from Power Plants
  - a. Disposal of ash is not authorized by this permit.
  - b. This permit does not pertain to permits for disposal of ash or exemptions for beneficial uses of ash under the Missouri Solid Waste Management Law and regulations.
  - c. This permit does not authorize off-site storage, use or disposal of ash in regard to water pollution control permits required under 10 CSR 20-6.015 and 10 CSR 20-6.200.
  - d. Subsurface discharges from wastewater treatment ponds or ash ponds shall, at the property boundary, meet the effluent limitations for subsurface waters of the state under 10 CSR 20-7.015(7), with appropriate consideration of up-gradient water quality.
- 13. Permittee is exempt from Clean Water Act section 311 reporting for sulfuric acid and sodium hydroxide as per 40 CFR 117.12.

14. Whole Effluent Toxicity (WET) tests shall be conducted as follows:

WET test samples shall be collected during a period of time when biocide residuals will be present in the effluent.

At the Ameren, UE Sioux Plant, Whole Effluent Toxicity (WET) tests will be required for Outfall #001 only if biocides are used. The WET test will only be required in the first year if the initial test passes. If the WET test does not pass in the first year, the test must be run <u>annually for the duration of the permit</u> or until biocide use is discontinued.

An initial WET test will be required for Outfall #002 (Ash Pond) and #006 (New Ash Pond). The WET test will only be required in the first year if it passes at all effluent concentrations. If the WET test does fail at any concentration in the first year, the test must be run annually for the duration of the permit.

(PRIVATE) SUMMARY OF WET TESTING FOR THIS PERMIT							
OUTFALL	A.E.C. %	C. % FREQUENCY SAMPLE TYPE MONTH					
Outfall #001	66%	Annually	grab	January			
Outfall #002, #006	10%	Annually	grab	January			

- (a) Test Schedule and Follow-Up Requirements
  - (1) Perform a single-dilution test in the months and at the frequency specified above. If the effluent passes the test, do not repeat the test until the next test period. Submit test results along with complete copies of the test reports as received from the laboratory within 30 calendar days of availability to the
  - (2) If the effluent fails the test, a multiple dilution test shall be performed within 30 calendar days , and biweekly thereafter, until one of the following conditions are met:

WPCP, Water Quality Section, P.O. Box 176, Jefferson City, MO 65102.

- (a) THREE CONSECUTIVE MULTIPLE-DILUTION TESTS PASS. No further tests need to be performed until next regularly scheduled test period.
- (b) A TOTAL OF THREE MULTIPLE-DILUTION TESTS FAIL.
- (3) The permittee shall submit a summary of all test results for the test series along with complete copies of the test reports as received from the laboratory to the WPCP, Planning Section, P.O. Box 176, Jefferson City, MO 65102 within 14 calendar days of the third failed test.
- (4) Additionally, the following shall apply upon failure of the third test: A toxicity identification evaluation (TIE) or toxicity reduction evaluation (TRE) is automatically triggered. The permitee shall contact WPCP, Planning Section to ascertain as to whether a TIE or TRE is appropriate. The permittee shall submit a plan for conducting a TIE or TRE to the Planning Section of the WPCP within 60 calendar days of the date of DNR's direction to perform either a TIE or TRE. This plan must be approved by DNR before the TIE or TRE is begun. A schedule for completing the TIE or TRE shall be established in the plan approval.

- 14. Whole Effluent Toxicity (WET) (continued)
  - (a) Test Schedule and Follow-Up Requirements (continued)
    - (5) Upon DNR's approval, the TIE/TRE schedule may be modified if toxicity is intermittent during the TIE/TRE investigations. A revised WET test schedule may be established by DNR for this period.
    - (6) If a previously completed TIE has clearly identified the cause of toxicity, additional TIEs will not be required as long as effluent characteristics remain essentially unchanged and the permittee is proceeding according to a DNR approved schedule to complete a TRE and reduce toxicity. Regularly scheduled WET testing as required in the permit, without the follow-up requirements, will be required during this period.
    - (7) All failing test results shall be reported to WPCP, Planning Section, P.O. Box 176, Jefferson City, MO 65102within 14 calendar days of the availability of the results.
    - (8) When WET test sampling is required to run over one DMR period, each DMR report shall contain information generated during the reporting period.
    - (9) Submit a concise summary of all test results with the annual report.
  - (b) PASS/FAIL procedure and effluent limitations:
    - (1) To pass a single-dilution test, mortality observed in the AEC test concentration shall not be significantly different (at the 95% confidence level; p = 0.05) than that observed in the upstream receiving-water control sample. The appropriate statistical tests of significance will be those outlined in the most current USEPA acute toxicity manual or those specified by the MDNR.
    - (2) To pass a multiple-dilution test:
      - (a) the computed percent effluent at the edge of the zone of initial dilution, Acceptable Effluent Concentration (AEC), must be less than three-tenths (0.3) of the  $LC_{50}$  concentration for the most sensitive of the test organisms; or,
      - (b) all dilutions equal to or greater than the AEC must be nontoxic. Failure of one multiple-dilution test is an effluent limit violation.
  - (c) Test Conditions
    - (1) Test Type: Acute Static non-renewal
    - (2) Test species: Ceriodaphnia dubia and Pimephales promelas (fathead minnow). Organisms used in WET testing shall come from cultures reared for the purpose of conducting toxicity tests and cultured in a manner consistent with the most current USEPA guidelines. All test animals shall be cultured as described in the most current edition of Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.
    - (3) Test period: 48 hours at the "Acceptable Effluent Concentration" (AEC) specified above.

- 14. Whole Effluent Toxicity (WET) (continued)
  - (c) Test Conditions (continued)
    - (4) When dilutions are required, upstream receiving stream water shall be used as dilution water. If upstream water is unavailable or if mortality in the upstream water exceeds 10%, "reconstituted" water will be used as dilution water. Procedures for generating reconstituted water will be supplied by the MDNR upon request.
    - (5) Single-dilution tests will be run with:
      - (a) Effluent at the AEC concentration;
      - (b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
      - (c) reconstituted water.
    - (6) Multiple-dilution tests will be run with:
      - (a) 100%, 50%, 25%, 12.5%, and 6.25% effluent, unless the AEC is less than 25% effluent, in which case dilutions will be 4 times the AEC, two times the AEC, AEC, 1/2 AEC and 1/4 AEC;
      - (b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
      - (c) reconstituted water.
    - (7) If reconstituted-water control mortality for a test species exceeds 10%, the entire test will be rerun.

## SUMMARY OF TEST METHODOLOGY FOR WHOLE-EFFLUENT TOXICITY TESTS

Whole-effluent-toxicity test required in NPDES permits shall use the following test conditions when performing single or multiple dilution methods. Any future changes in methodology will be supplied to the permittee by the Missouri Department of Natural Resources (MDNR). Unless more stringent methods are specified by the DNR, the procedures shall be consistent with the most current edition of Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms,

Test conditions for Ceriodaphnia dubia:

Test duration: 48 h

Temperature: 25  $\pm$  1°C Temperatures shall not deviate by more

than 3°C during the test.

Light Quality: Ambient laboratory illumination

Photoperiod: 16 h light, 8 h dark Size of test vessel: 30 mL (minimum)

Size of test vessel:

Volume of test solution:

Age of test organisms:

30 mL (minimum)

15 mL (minimum)

<24 h old

No. of animals/test vessel: 5
No. of replicates/concentration: 4

No. of organisms/concentration: 20 (minimum)

Feeding regime: None (feed prior to test)

Aeration: None

Dilution water: Upstream receiving water; if no upstream flow,

synthetic water modified to reflect effluent

hardness.

Endpoint: Pass/Fail (Statistically significant Mortality

when compared to upstream receiving water

control or synthetic control if upstream water

was not available at  $p \le 0.05$ )

Test acceptability criterion: 90% or greater survival in controls

Test conditions for (Pimephales promelas):

Test duration: 48 h

Temperature: 25  $\pm$  1°C Temperatures shall not deviate by more

than 3°C during the test.

Light Quality: Ambient laboratory illumination

Photoperiod: 16 h light/ 8 h dark Size of test vessel: 250 mL (minimum) Volume of test solution: 200 mL (minimum)

Age of test organisms: 1-14 days (all same age)

No. of animals/test vessel: 10

No. of replicates/concentration: 4 (minimum) single dilution method

No. of organisms/concentration: 2 (minimum) multiple dilution method 40 (minimum) single dilution method

20 (minimum) multiple dilution method None (feed prior to test)

Aeration: None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min.

Dilution water: Upstream receiving water; if no upstream flow, synthetic water

modified to reflect effluent hardness.

Endpoint: Pass/Fail (Statistically significant Mortality

when compared to upstream receiving water

control or synthetic control if upstream water

was not available at  $p \le 0.05$ )

Test Acceptability criterion: 90% or greater survival in controls